GREEN SYNTHESIS AND CHARACTERIZATION OF TIO_2 NANOPARTICLES USING *PROSOPIS CINERARIA* LEAF EXTRACT AND ITS PHOTOCATALYTIC, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES

Synopsis

For Registration for Ph. D Programme

By

VAANI YADAV

UNDER THE SUPERVISION OF

Prof. (Dr.) Varsha Gupta

Professor & Head

Department of Microbiology

JECRC University, Jaipur

CERTIFICATE

This is to certify that the research proposal submitted by **VAANI YADAV** (Registration no**: Ph.D./21/10129**), entitled "GREEN SYNTHESIS AND CHARACTERIZATION OF TiO_2 NANOPARTICLES USING *PROSOPIS CINERARIA* LEAF EXTRACT AND ITS PHOTOCATALYTIC, ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES" in fulfilment for the award of Doctor of Philosophy. She is submitting her synopsis for Ph.D. under my guidance and in supervision and I have gone through the content presented in the report.

Date:

Prof. (Dr.) Varsha Gupta
Professor& Head
Department of Microbiology
JECRC, University, Jaipur

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ABSTRACT:

Nanotechnology involves novel strategies with the help of which atoms and nanoparticles (key product) are manipulated. These nanoparticles have dimensions ranging from 1–100 nanometers (Farias *et al*., 2014) and are more reactive because of their larger surface area per unit volume ratio. Technology based on the nanoparticles helps in improving efficiency, sustainability and speed up the already existing processes (Xu *et al*., 2018). TiO_2 nanoparticle has been studied extensively due to its important properties such as photo-catalytic, antibacterial, antioxidant, and they are less toxic in nature etc. *Prosopis cineraria* have pharmacological activities such as antioxidant, anti- bacterial, anti-hypercholesterolemia, antitumor, analgesic and antipyretic, anti-hyper-glycemic (Kulshreshtha *et al*., 2019). *Prosopis cineraria* is an excellent source of herbal medicine due to the presence of phytochemicals like alkaloids, proteins, carbohydrates, flavonoids, saponins and tannins. Medicinal efficacy of *Prosopis cineraria* extract with titanium oxide nano-particle will be studied in this work. Leaf extract is used as capping and reducing agent. Chemical synthesis of nanoparticle requires high cost and is more toxic. So, the nanoparticles of titanium oxide will be synthesized using *Prosopis cineraria* leaf extract by green route method. Phytochemicals are present in leaves which are capable of reducing metal salts into metal nanoparticles which helps in enhancing its activity and increases its tendency for better percolation. The tendency of *Prosopis cineraria* with dyes and its degradation efficiency will also be studied.

Keywords: *Prosopis cineraria*; *nanoparticles; titanium oxide; antibacterial activity; anti-oxidant activity; photo-catalytic activity; dyes.*

INTRODUCTION:

Nanotechnology is an enabling technology that allows us to develop materials with improved or totally new properties. Nanotechnology plays an important role in significant technologies via nanoparticles such as drug-gene delivery, chemical industry, optoelectronic devices, biomedical sciences, mechanics, catalysis, space industries and photo electrochemical applications (Singh *et al*., 2019). Nanoparticles are the emerging edge in developing field of nanotechnology. Nanoparticles have a unique size- dependent property. Due to this property it makes the particles superior in many areas of human activity (Salata, 2004). Nanoparticle is a unit dimension which is measured in nanometres (1 nanometer = 10^{-9} metre). The size of the nanoparticles is very small due to this they can easily enter in biological tissue. To increases the properties or for obtaining composite materials they are mixed with other materials. Nanoparticles contribute to cleaner, lighter and smarter surfaces and systems. Nanoparticles have more surface area per area unit volume ratio. This leads to nanoparticles being more reactive. They can easily react with active substances to reach a relatively stable state (Xu *et al*., 2018). The technologies based on nanoparticles helps in enhancing the efficiency and sustainability of our environment. It helps in boosting or speeds up the previously existing processes. Nanomaterials are also being used in medical field as in drug delivery and gene therapy, separation of biological cells and molecules and their purification, fluorescent biological labels, sunscreens, paints, imaging (Salata, 2004).

Titanium oxide nanoparticles have been found to have good cytotoxic, antimicrobial and photo-catalytic potentials and are non-toxic and stable. They are widely used in industries such as in cosmetic industry, electrochemical devices, pollution control and antimicrobial coatings, catalysts, sunscreens etc. (Amarnath *et al*., 2013). Catalytic process, gas sensors, solar cells and high temperature super-conductors like modern technologies have also benefitted from titanium oxide NPs (Schilling *et al*., 2010). TiO_2 is a versatile material having greater physical stability that plays an important role in a various applications. It is also non-toxic in nature (Weir *et al*., 2012). TiO_2 has been found to have a large surface area, high catalytic activity, low rate of electron-hole pair recombination and high molecular oxygen adsorption capacity (Zeman and Takabayashi, 2003). TiO_2 nano-catalysts are wide-band gap semiconductors having excellent photo-catalytic activity under UV irradiation (Khade *et al*., 2015).

In ancient times medicinal plants have been used traditionally used because of their good therapeutic effects on humans. In pharmaceutical industries medicinal plants are attaining an

equitable place. These medicinal plants have a great nutritional value and compounds present in plants helps in treating many diseases (Badami *et al*., 2001). Due to presence of bioactive phyto-chemical in the medicinal plants, they help in curing many diseases (Nostro *et al*., 2000).

"Khejri" is the botanical name of *Prosopis cineraria*. Khejri is state tree of Rajasthan. It is also known as "Wonder tree" or "Golden tree" of the desert (Pareek *et al*., 2015). In Indian traditional system *Prosopis cineraria* known to be thorny, medium sized, irregular branched tree. It comes under Fabaceae family (Yadav *et al*., 2018). 44 species of *Prosopis* genus so far identified and also called as jand, shami, khijda and khejri (Henciya *et al*., 2017). It is also called as kalpataru (a wish granting tree) because every part of this plant is used for medicinal purpose. It is commonly found in hot arid and semi-arid regions of the world (Pasiecznik *et al*., 2001). Broad spectrum ailments like leucoderma, mouth ulcers, eye diseases, leprosy, asthma, boils and rheumatism can be treated with the help of this plant by traditional healers (Robertson *et al*., 2011). *Prosopis* extracts have many biological properties like as antibacterial, anthelmintics, antihyperglycemic, antioxidant, antitumor, and anticancer (Zhong *et al*., 2022). High nutrient contents like fat, carbohydrate, protein are found in the leaf of the tree having antioxidant, anti hyperglycemic and antibacterial activities. Flowers of *Prosopis cineraria* help in curing skin diseases and act as blood purifier. The bark of this tree is also useful as it is used in treating many diseases like asthma, cough, dysentery and tremors of the muscles (Chahar, 2019). Phytochemicals like proteins, flavanoids, tannins, alkaloids, saponins are found in *Prosopis cineraria*. Many chemical constituents are found in *Prosopis cineraria* such as Gallic acid, ferulic, caffeic acid and hydroxybenzoic acid leuteolic etc. These are the main constituents of *Prosopis cineraria* (Kumari and Solanki, 2021).

Taxonomic classification of *Prosopis cineraria*: - (Based on Bentham and Hoocker classification, 1883)

Kingdom: - Plantae
Sub Kingdom: - Phanerogames
Division: - Angiosperms
Class: - Dicotyledons
Sub-class: - Polypetalae
Series: - Calyciflorae
Order: - Rosales
Family: - Leguminosae (Fabaceae)

Genus: - *Prosopis*

Species*: - cineraria*

Source: file:khejri.jpg Wikipedia Commons

Fig 1: Tree of *Prosopis cineraria* in Desert of Rajasthan

Source: Green-India-Shami-Tree-Banni-SDL625224249-3-f4777

Fig 2: Leaves of *Prosopis cineraria*

Biological methods have many advantages as compared to chemical methods, as biological methods are more benignant, cost effective, more biocompatible and eco-friendly. The microorganisms also play an important role in reducing and stabilizing metal nanoparticles. Metal ions got trapped on the surface of microbial cells and then reduce to nanoparticles due to presence of enzymes.

Role of plants in green synthesis of nanoparticles

"Green chemistry" is a biosynthetic route which includes microorganisms like as bacteria, algae, fungi, plants etc. Synthesis of nanoparticles is safe, environment friendly, non- toxic and biocompatible green approach to synthesize nanoparticles for various medical and biological applications (Duan *et al*., 2015). Plants need low maintenance and are cost effective, so this is the advantage of synthesizing nanoparticles with plants (Hulkoti and Taranath, 2014). By adopting green approach nanoparticles such as oxides of titanium, iron, gold, silver, zinc have been easily synthesized (Singh *et al*., 2018). Nanoparticles synthesized from plants have more advantage as compared to other biosynthetic chemical nanoparticles. Almost all the parts of

the plants such as fruit, seeds, leaf, root and stem are used in synthesis of nanoparticles (Iravani, 2011). Plant extract contains phytocompounds such as terpenoids, polyphenols and polyols which helps in metallic ion reduction (Ovais *et al*., 2018). Phyto-chemicals are present in the plant extract which behaves like stabilizing and reducing agent. Almost every part of this plant can be used for synthesis of nanoparticles (Narayanan and Sakthivel, 2011)

Green synthesis of nanoparticles using plants is developing positive effects on the environment. Long term effects on human beings as well as on animal need to be concerned and should be resolved by green route synthesis of nanoparticles and its applications should be increased exponentially for environment sustainability and development (Jaduon *et al*., 2021). Green synthesis mainly focuses at reducing the use of toxic chemicals. Plants contain reducing and capping agents. Nanotechnology and green chemistry combined to produce environment friendly nanoparticles via plants and microbes to reduce the problem of toxicity (Lateef *et al*., 2016).

TiO_2 NPs can be synthesized by using green route method by adopting various methods. TiO_2 NPs are widely used in biomedical fields as well as in clinical fields. Their bioactivity depends on the shape and size of the nanoparticles. Because of electrostatic force and nano-sized pores in the membrane, they can reach out with bacterial cellulose. Additionally, nanoparticles also can interrupt normal bacterial cellular respiration and can cause disruption in DNA and proteins (Sathiyavimal *et al*., 2021).

Nanoparticles are attainable alternative to antibiotics and have high potential to solve the emergence of bacterial drug resistance. NPs are utilized in anti-bacterial coatings for implantable devices to prevent bacterial infection (Rudramurthy *et al*., 2016).

So, the green route synthesis of TiO_2 NPs by using of *Prosopis cineraria* leaf extract will be used. The *Prosopis cineraria* plant is rich in phyto-compounds which help to increase its biological properties of NPs and helps in reducing toxicity of the materials. The tendency of *Prosopis cineraria* with dyes and its degradation efficiency will also be examined.

REVIEW OF LITERATURE:

Prosopis cineraria contains alkaloids, flavanoids and compounds such as 5- isorhamnetin-3-diglucoside, hydroxytryptamine, apigenin, l- arabinose, quercetin, tannin and tryptamine

(Rastogi and Mehrotra, 1993). Seeds of *Prosopis cineraria* contains non- glycosidic polyhenolics, luteolin, gallic acid and prosogerin- E, patulitrin, rutin and glycosidic polyphenolics. Seeds have been also reported containing fatty acids like stearic acid, oleic acid, palmitic acid and have sterols such as stimasta1,3,5-triene, stigmasterol, β- sitosterol, etc. (Bhardwaj *et al*., 1981). Leaves and pods were also found to be having amino acids like glutamic acid, serine, aspartic acid, cysteine, alanine, leucine, histidine isoleucine, proline, valine, threonine, and phenylalanine (Mazzuca and Balzaratti, 2003).

Prosopis cineraria have various pharmacological activities such as antioxidant, anti-hypercholesterolemia, antitumor, analgesic and antipyretic, anti-hyper-glycemic (Kulshreshtha *et al*., 2019). *Prosopis cineraria* are excellent source of herbal medicine. In alcohol phyto-chemicals like alkaloids, flavonoids, saponins and tannins found to have a great and positive response against *Prosopis cineraria*. Extract of this plant is used to kill intestinal parasitic worms in humans. Leaves of *Prosopis cineraria* also contain insecticidal and fungicidal properties. Eye problems can cure by smoke of burned leaves of *Prosopis cineraria* (Malik and Kalidhar, 2007). This plant is rich in antioxidant compounds which aids in reducing the probability of heart diseases and cancer. These antioxidants are good for human health that was found in this plant (Muhammad Fazal, 2021). 2% pre-concentrated *Prosopis* bark extract was used to formulate a stable phyto-cosmetic emulsion system to revive facial skin properties. It was reported that ethanol bark extract is an excellent source of flavanoids and phenolic compound having antioxidant potential (Mohammad, *et al*., 2018). *Prosopis cineraria* leaves extracts were treated with different reagents and different proteins were screened out and characterized by NMR, IR and mass spectroscopy. Antimicrobial activity against *Staphylococcus aureus*, *Aspergillus tubigensis* and *Pseudomonas ruginosa* were explored. Antimicrobial activity is determined by disc diffusion method by observing zone of inhibition. *Pseudomonas aureginosa* was found to have maximum antimicrobial activity (Kulshreshtha *et al.*, 2019).

Green route synthesis of titanium oxide nanoparticles has been found to have good antioxidants and antibacterial activities. TiO_2 found to have antimicrobial activity when bring to UV light or sunlight due to its strong oxidizing property (Kumar *et al*., 2014). The bio synthesis of TiO_2 nanoparticles using *Acorus calamus* plant leaf extract was performed, antimicrobial action against both gram positive and gram negative were found having zone of inhibition and degradation efficiency was found 96.59% against rhodamine B (Ansari *et al*., 2022). Green synthesis of titanium dioxide nanoparticles by using *Azadirachta indica* was done. It was found

that bioactive compounds were present in leaf extract which are responsible for the reducing metal ions into nanoparticles. The NP's were found to have broad spectrum antimicrobial activity and their antibacterial effects were found more effective in case of TiO_2 nanoparticles rather than TiO_2 compound (Thakur *et al*., 2014). TiO_2 NP were synthesised using *Cola nitida* (nut tree) using leaf, pod and seed shell nanoparticles characterization was done to examine the antimicrobial, antioxidant, anticoagulant and dye degradation activities. Involvement of phenolic compounds and proteins in the photosynthesis of TiO_2 NPs were found. TiO_2 NPs inhibits multidrug resistant bacteria and toxigenic fungi. They were found to have efficient dye degradation, DPPH and H_2O_2 scavenging activities. They also help in reduction of blood coagulation in humans. It indicates that the TiO_2 NPs can be very helpful in catalytic processes as well as in biomedical applications (Akinola *et al*., 2020)

WHY I CHOOSE TO DO RESEARCH ON *PROSOPIS CINERARIA*?

Prosopis cineraria is considered as one of the medicinal plants and rich source of therapeutic agents. It helps in treatment of various ailments without producing any side effects. It has many health benefits as well as nutritional values. It can be used as alternative for the conventional legumes and can be used as a source of gluten free products. Pods of this plant in a dried form are consumed as vegetable which can add value in food product development and in biomedical field. It plays an important role in socio-economic growth of villages of arid regions. It increases soil fertility, provides timber, fuel and fodder. Biosynthesized nano-particle can be used in medicine as therapeutic agents but it depends on its antibacterial activity. TiO_2 NP synthesised by using *Prosopis cineraria* leaf extract has potential value in the field of biomedical applications and also eco-friendly as well as non-toxic in nature. As a photo-catalyst, TiO_2 is extensively used in many environmental and energy applications due to its efficient photo-activity, high stability, low cost and safety to the environment and human beings. This study will be conducted to check the bioactivity i.e. antibacterial, antioxidant and photo-catalytic activities of such phytochemical compounds of *Prosopis cineraria* with the TiO_2 .

RESEARCH OBJECTIVES:

- Biosynthesis of titanium-oxide nanoparticles using phyto-compounds from *Prosopis cineraria* leaf extract.
- Phytochemical screening of *Prosopis cineraria* leaf extract.

- Characterisation of titanium nanoparticles using Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) etc.
- To study the antibacterial and antioxidant activity of synthesized titanium nanoparticle.
- To identify bactericidal properties of synthesized nanoparticle using Live/ Dead cell viability assay.
- To check its photo-catalytic activity with Congo red and Methylene blue dyes prominently used in dyeing industries.

RESERCH METHODOLOGY:

➢ Materials and methods:

a) **Preparation of leaf extract**:

Fresh *Prosopis Cineraria* plant leaves will be collected from Jaipur, Rajasthan. Then wash the leaves and will be dried at room temperature for 7 days. Dried leaves will be cut into fine pieces and grinded to get its powdered form. Leaf extract will be prepared from powder with the help of soxhlet apparatus. Ethanol will be used as a solvent. The leaf extract will be filtered through the Whatman filter paper (No. 1) and then it will be stored in the cooled room for further studies (Patidar and Jain, 2017).

b) **Phytochemical screening of leaf extract:** The plant extract obtained from the soxhlet apparatus will be used for analyzing various phytochemical constituents like alkaloids, carbohydrates, proteins, saponins, starch, flavanoids, tannins, steroids and phenolic compounds and quantitative analysis will be performed (Pathak and Kumar, 2017).

c) **Biosynthesis of titanium-oxide nanoparticles:**

Biosynthesis of titanium-oxide nanoparticles will be prepared by using TTIP (titanium tetra isopropoxide), $TiCl_4$ (Titanium tetrachloride), $TiO(OH)_2$ (metatitanic acid or titanyl

hydroxide), and $TiOSO_4$ (titanium oxysulphate) precursors. TiO_2 particles will be dissolved in ethanol or distilled water and then plant extract will be added in the mixture, drop by drop. After that, the solution will be stirred continuously and change in color will indicate the formation of nanoparticles. Again NP will be centrifuged and collect the precipitate. The precipitate will be washed with distilled water to remove impurities. The separated titanium oxide NP's will be dried and calcinations will be done in a furnace temperature ranging 400-800℃ for 5 hours. Then, calcinated titanium oxide nano powder will be used for futher processes (Verma *et al*., 2022).

d) **Characterisation of Titanium oxide NP's:**

Biosynthesized TiO_2 NPs will be confirmed by UV–Visible spectroscopy. FT-IR will be used to identify the functional groups of TiO_2 NP's. The crystalline phase of the biosynthesized TiO_2 NPs will be determined via X-Ray diffraction (XRD). The surface morphological characterization of the biosynthesized NPs. TiO_2 will be imaged by using of Field Emission Scanning Electron Microscope (FE-SEM). The particle size of TiO_2 NPs will be studied by using a High Resolution Transmission Electron Microscope (HR-TEM) (Verma *et al*., 2022).

e) **Bactericidal activity of TiO_2 NP's:**

The biosynthesized TiO_2 NPs will be identified for their antagonistic activity against the clinical pathogenic bacteria of G+ and G−strains by using agar well diffusion method. Overnight bacterial inoculums will be prepared in the nutrient broth and evenly spread on the Mueller Hinton Agar (MHA) containing plates. The sterile wells will be created into the MHA plates and the biosynthesized TiO_2 NP's will be added to the wells. All the inoculated Petri plates will be maintained at 37℃ for 24 hr. Bactericidal activity of zones of inhibition will be measured (Kumar *et al*., 2014).

e) **Antioxidant activity of TiO_2 NP's:**

DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging activity measures the free radical activity. The radical scavenging and antioxidant potential of the plant extracts will be determined by the ability of plant extracts to scavenge the stable free radical of

DPPH and transform it into 2, 2- diphenyl-1-picryl- hydrazine. 1 ml of DPPH prepared in methanol will be added into the sample containing TiO_2 NP's. The degree of de-colorization from purple to yellow color will be measured spectro-photometrically. The reaction mixture will be mixed well and left in dark at room temperature for 30 minutes. The absorbance will be measured spectro-photometrically at 517 nm. The scavenging ability of the plant extract will be calculated using this equation;

DPPH Scavenging activity (%) = [(Abs control −Abs sample)] / (Abs control)] ×100

Where; Abs control is that the absorbance of DPPH + methanol; Abs sample is that the absorbance of DPPH radical + sample (i.e. extract) (Kumar *et al*., 2014).

f) **Live or dead cell viability assay:**

The bactericidal properties of biosynthesized TiO_2 NPs will be checked by using Confocal Laser Scanning Microscopic (CLSM). The cellular interaction of both G+ and G− bacteria will be visualized by CLSM. When bacterial growth will attain mid log phase, then ultimately the final concentration of TiO_2 NPs will be added. Incubate it after it will reach to the later log phase. The treated bacterial cultures will be centrifuged. The pellets will be retrieved and then put back into suspension with 1X PBS (Phosphate Buffered Saline) solution. The process will be again repeated two times for obtaining the samples in pure form. After that each bacterial pellet will be individually collected and merge or assorted with staining dyes of Acridine orange (interact with RNA of a bacterial cell/ live cells) and Ethidium bromide (interacts with DNA/only penetrates into dead or deceased bacterial cells due to disruption in cell wall of bacteria) and then incubate it. The test bacterial samples will be fixed on the crystal clear glass slides. Live cells will be imaged green and dead cells will be imaged red colored cells and will be visualized using CLSM (Sagadevan *et al*., 2021).

g) **Photo-catalytic activity:** The biosynthesized TiO_2 NP's photo-catalytic activity will be studied against the dyes used in dyeing industries. Congo red and Methylene blue dyes will be used in this study. TiO_2 and dye containing aqueous solution will be used and will be placed in dark condition. Subsequently irradiation between different time intervals will be noted. After then, centrifugation will be done and the effective dye degradation rate will be examined by using UV-Visible Spectroscopy

Degradation Efficiency (%) = $(Ao - A_t / Ao) \times 100$

Where, Ao is initial dye concentration, A_t is absorbance of the solution after illumination at time 't' after different time intervals; % is the rate of dye molecules degradation, respectively (Ansari *et al*., 2022).

EXPECTED OUTCOME:

- Titanium oxide nanoparticles by using *Prosopis cineraria* leaf extract will be synthesized.
- This study will be conducted to synthesis cost effective, reproducible and more efficient TiO_2 NPs.
- *Prosopis Cineraria* leaves could be the best associated with potential for beneficial integration in food and pharmaceutical industry.

PLAN OF WORK:

Research Plan of Minimum TimeLine

* As approved Extension

Research Activity	6	12	18	24	30	36	*
1. Course Work							
2. Literature Survey							
3. Methodology Steps							
4. Quarterly Reports							
5. Communication of Papers							
6. Preparation of Final Thesis							
7. Submission of Thesis							

LIMITATIONS OF THE STUDY:

This study will be performed with the aim of green biosynthesis of titanium nanoparticles by using *Prosopis cineraria* and its characterisation to study its antibacterial, antioxidant and photo-catalytic activity. *Prosopis cineraria* have many properties like anti-hyperglycaemic, anti-tumour, anti-cancer and antidepressant activities but this study limited to examine antibacterial, antioxidant and photo-catalytic activity of biosynthesized titanium oxide nanoparticles.

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